Ram Semen Processing and Cryopreservation Protocol for Laparoscopic Insemination Use

The concentration and motility of the semen sample are determined using spectrophotometry and a Hamilton Thorne motility analyzer (Beverly, MA), respectively (at least 7 fields of analysis and 1000 cells). Semen samples are diluted to 200 x 10^6 sperm/mL in egg yolk-Tris media (see recipe below).

The samples are then cooled to 5°C over 2 to 2.5 hours and loaded into 0.5ml straws. The samples are frozen using the Cryo Bio System Mini Digitcool UJ400 (IMV Corporation, Minneapolis, MN) with the following curve: 5°C to -5°C at -4°C per minute, -5°C to -110°C at -25°C per minute, -110°C to -140°C at -35°C per minute, and plunged into liquid nitrogen for storage.

Cryopreserved samples are thawed for 30 seconds in a 37°C water bath and analyzed for motility as described previously.

Semen Cryopreservation Media Recipe from Sanchez-Partida et al., 1998:

300mM Tris

28mM glucose

95mM citric acid

5% glycerol (by volume)

15% egg yolk

1mg/ml streptomycin sulfate

0.06mg/ml benzylpenicillin

Laparoscopic insemination:

The estrous cycles of ewes are synchronized using either:

Sponges for 14 days (e.g. Chronogest CR containing 40 mg fluorogestone acetate, Intervet, Milton Keynes, UK) and then given PMSG (400 IU, i.m.; total volume = 4mL from an 18 gauge needle; single injection) 48 hours prior to sponge removal;

-or-

CIDRs (e.g. 0.3 g progesterone in an inert silicone elastomer for 12 days; Pfizer Animal Health, New York, NY) with PMSG (400 IU i.m. as described previously).

The ewes are restrained in dorsal recumbency and inseminated laparoscopically 56 hours after CIDR removal.

Reference:

Sanchez-Partida, L.G., Setchell, B.P., and Maxwell, W.M.C. Effect of compatible solutes and diluent composition on the post-thaw motility of ram sperm. Reprod. Fertil. Dev. 1975; 10, 347-357.

Originally created April 2004, modified September 2014